

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a fusion promoter said fusion promoter comprising at least one promoter that is modified to have altered activity in at least one gram-positive organism said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *lacO*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter.
2. The isolated fusion promoter of Claim 1, wherein said at least one promoter is selected from the group consisting of SEQ ID NOs.: 36-45.
3. The isolated nucleic acid of Claim 1, wherein said at least one operator is *xylO*.
4. The isolated nucleic acid of Claim 3, wherein said at least one promoter is T5.
5. The isolated nucleic acid of Claim 3, further comprising a second operator.
6. The isolated nucleic acid of Claim 5, wherein said second operator is *lacO*.
7. The isolated nucleic acid of Claim 1, wherein said fusion promoter is responsive to an inducer.
8. The isolated nucleic acid of Claim 7, wherein said inducer is xylose.
9. The isolated nucleic acid of Claim 1, wherein said fusion promoter is titratable.
10. The isolated nucleic acid of Claim 1, wherein said at least one gram-positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.

11. The isolated nucleic acid of Claim 1, wherein said at least one gram-positive organism is *Staphylococcus aureus*.

12. The isolated nucleic acid of Claim 1, wherein said at least one gram-positive organism is *Enterococcus faecalis*.

5 13. An isolated nucleic acid comprising a fusion promoter said fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59, P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *lacO*, wherein said at least one operator is positioned such that binding of at least one repressor to said at  
10 least one operator represses transcription from said fusion promoter.

14. The isolated nucleic acid of Claim 13, wherein said at least one operator is *xylO*.

15. The isolated nucleic acid of Claim 14, wherein said at least one promoter is T5.

15 16. The isolated nucleic acid of Claim 14, further comprising a second operator.

17. The isolated nucleic acid of Claim 16, wherein said second operator is *lacO*.

18. The isolated nucleic acid of Claim 13, wherein said fusion promoter is  
20 responsive to an inducer.

19. The isolated nucleic acid of Claim 18, wherein said inducer is xylose.

20. The isolated nucleic acid of Claim 13, wherein said fusion promoter is titratable.

21. The isolated nucleic acid of Claim 13, wherein said at least one gram-  
25 positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus*

*epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.

22. The isolated nucleic acid of Claim 13, wherein said at least one gram-positive organism is *Staphylococcus aureus*.

5 23. The isolated nucleic acid of Claim 13, wherein said at least one gram-positive organism is *Enterococcus faecalis*.

24. An isolated fusion promoter comprising one of SEQ ID NO.: 26-35.

25. A vector comprising the isolated nucleic acid of Claim 1.

26. A vector comprising the isolated nucleic acid of Claim 13.

10 27. A vector comprising the isolated nucleic acid of Claim 24.

28. The vector of Claim 25 further comprising at least one replicon selected from the group consisting of p15a, pC194 and pCT1138.

29. The vector of Claim 25 further comprising a reporter gene operably linked to said fusion promoter.

15 30. The vector of Claim 29, wherein said reporter gene is *lacL-lacM*.

31. The vector of Claim 30, wherein *lacL-lacM* is derived from *Leuconostoc mesenteroides*.

32. The vector of Claim 25, wherein said at least one operator is *xyIO*.

33. The vector of Claim 32, wherein said at least one promoter is T5.

20 34. The vector of Claim 32, further comprising a second operator.

35. The vector of Claim 34, wherein said second operator is *lacO*.

36. The vector of Claim 25, wherein said fusion promoter is responsive to an inducer.

37. The vector of Claim 36, wherein said inducer is xylose.

25 38. The vector of Claim 25, wherein said fusion promoter is titratable.

39. The vector of Claim 25, wherein said at least one gram-positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*,  
30 *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium*

*tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.

5        40. The vector of Claim 25, wherein said at least one gram-positive organism is *Staphylococcus aureus*.

41. The vector of Claim 25, wherein said at least one gram-positive organism is *Enterococcus faecalis*.

42. The vector of Claim 25, further comprising a random fragment of a microbial genome operably linked to said fusion promoter.

10       43. The vector of Claim 25, further comprising a nucleic acid that encodes a peptide, wherein said nucleic acid is operably linked to said fusion promoter.

44. The vector of Claim 25, further comprising a nucleic acid that is complementary to a portion of a microbial genome, wherein said nucleic acid is operably linked to said fusion promoter.

15       45. The vector of Claim 25, further comprising a nucleic acid that encodes a molecule that inhibits the proliferation of microbe, wherein said nucleic acid is operably linked to said fusion promoter.

46. A host cell comprising the nucleic acid of Claim 1.

47. A host cell comprising the nucleic acid of Claim 13

20       48. A method for identifying genes involved in cellular proliferation said method comprising the steps of:

25       (a) introducing into cells of a cell population a construct comprising an inducible fusion promoter operably linked to a nucleic acid, said fusion promoter comprising at least one promoter that is modified to have altered activity in at least one gram-positive organism said promoter being linked to at least one operator selected from the group consisting of *xytO*, *tetO*, *trpO*, *malO* and *λclO*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter;

(b) inducing transcription of said nucleic acid from said inducible fusion promoter;

(c) identifying the cells in said cell population whose proliferation is reduced in response to the induction of transcription of said nucleic acid; and

5 (d) identifying the gene from a cell identified in step (c) to which at least a portion of said nucleic acid is complementary.

49. A method for identifying genes involved in cellular proliferation said method comprising the steps of:

10 (a) introducing into cells of a cell population a construct comprising an inducible fusion promoter operably linked to a nucleic acid, said fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59, P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *λc1O*, wherein said at least one operator is positioned such that binding of at  
15 least one repressor to said at least one operator represses transcription from said fusion promoter;

(b) inducing transcription of said nucleic acid from said inducible fusion promoter;

20 (c) identifying the cells in said cell population whose proliferation is reduced in response to the induction of transcription of said nucleic acid; and

(d) identifying the gene from a cell identified in step (c) to which at least a portion of said nucleic acid is complementary.

50. The method of Claim 48, wherein said nucleic acid is random fragment of a microbial genome.

25 51. The method of Claim 48, wherein said nucleic acid encodes an aptamer.

52. The method of Claim 48, wherein said nucleic acid encodes a transcript that is complementary to a portion of a microbial genome.

30 53. The method of Claim 48, wherein said nucleic acid encodes a molecule that inhibits the proliferation of a microbe.

54. The method of Claim 48, wherein said at least one operator is *xylO*.
55. The method of Claim 54, wherein said fusion promoter is T5.
56. The method of Claim 54, wherein said fusion promoter further comprises a second operator.
- 5 57. The method of Claim 56, wherein said second operator is *lacO*.
58. The method of Claim 54, wherein said inducer is xylose.
59. The method of Claim 54, wherein said fusion promoter is titratable.
60. The method of Claim 48, wherein said at least one gram-positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.
- 10 61. The method of Claim 48, wherein said at least one gram-positive organism is *Staphylococcus aureus*.
62. The method of Claim 48, wherein said at least one gram-positive organism is *Enterococcus faecalis*.
- 15 63. A method for identifying genes involved in cellular proliferation said method comprising the steps of:
- (a) introducing into the genome of a cell a construct comprising an inducible fusion promoter operably linked to a nucleic acid, said fusion promoter comprising at least one promoter that is modified to have altered activity in at least one gram-positive organism said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter;
- 20 25

(b) comparing the proliferation of said cell cultured in the presence of a first concentration of an inducer that induces transcription from said fusion promoter with the proliferation of said cell cultured in the presence of a concentration of said inducer that is less than said first concentration, wherein a difference in proliferation indicates that said fusion promoter modulates a gene that is required for proliferation; and

(c) identifying the gene that is modulated by said fusion promoter.

64. A method for identifying genes involved in cellular proliferation said method comprising the steps of:

(a) introducing into the genome of a cell a construct comprising an inducible fusion promoter operably linked to a nucleic acid, said fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59, P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter;

(b) comparing the proliferation of said cell cultured in the presence of a first concentration of an inducer that induces transcription from said fusion promoter with the proliferation of said cell cultured in the presence of a concentration of said inducer that is less than said first concentration, wherein a difference in proliferation indicates that said fusion promoter modulates a gene that is required for proliferation; and

(c) identifying the gene that is modulated by said fusion promoter.

65. The method of Claim 63, wherein said nucleic acid is random fragment of a microbial genome.

66. The method of Claim 63, wherein said nucleic acid encodes an aptamer.

67. The method of Claim 63, wherein said nucleic acid encodes a transcript that is complementary to a portion of a microbial genome.

68. The method of Claim 63, wherein said nucleic acid encodes a molecule that inhibits the proliferation of a microbe.

69. The method of Claim 63, wherein said at least one operator is *xylO*.

70. The method of Claim 69, wherein said at least one promoter is T5.

5 71. The method of Claim 69, wherein said fusion promoter further comprises a second operator.

72. The method of Claim 71 wherein said second operator is *lacO*.

73. The method of Claim 63, wherein said inducer is xylose.

74. The method of Claim 63, wherein said fusion promoter is titratable.

10 75. The method of Claim 63, wherein said at least one gram-positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.

76. The method of Claim 63, wherein said at least one gram-positive organism is *Staphylococcus aureus*.

20 77. The method of Claim 63, wherein said at least one gram-positive organism is *Enterococcus faecalis*.

78. A method for identifying a compound which reduces the activity or level of a gene product required for proliferation of a cell said method comprising:

(a) introducing into a cell a construct comprising an inducible fusion  
25 promoter operably linked to a nucleic acid that is complementary to at least a portion of a proliferation-required gene, said fusion promoter comprising at least one promoter that is modified to have altered activity in at least one gram-positive organism said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *λc1O*,  
30 wherein said at least one operator is positioned such that binding of at least one



repressor to said at least one operator represses transcription from said fusion promoter;

(b) sensitizing said cell by inducing transcription from said fusion promoter;

5 (c) contacting said sensitized cell with a compound; and

(d) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which has not been sensitized.

79. A method for identifying a compound which reduces the activity or  
10 level of a gene product required for proliferation of a cell said method comprising:

(a) introducing into a cell a construct comprising an inducible fusion promoter operably linked to a nucleic acid that is complementary to at least a portion of a proliferation-required gene, said fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59,  
15 P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter;

(b) sensitizing said cell by inducing transcription from said fusion  
20 promoter;

(c) contacting said sensitized cell with a compound; and

(d) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which has not been sensitized.

25 80. The method of Claim 78, wherein said at least one operator is *xylO*.

81. The method of Claim 80, wherein said at least one promoter is T5.

82. The method of Claim 80, wherein said fusion promoter further comprises a second operator.

83. The method of Claim 82, wherein said second operator is *lacO*.

30 84. The method of Claim 78, wherein said inducer is xylose.

85. The method of Claim 78, wherein said fusion promoter is titratable.

86. The method of Claim 78, wherein said at least one gram-positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*,  
5 *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.

10 87. The method of Claim 78, wherein said at least one gram-positive organism is *Staphylococcus aureus*.

88. The method of Claim 78, wherein said at least one gram-positive organism is *Enterococcus faecalis*.

89. A compound identified using the method of Claim 78.

15 90. A method for inhibiting the activity or expression of a gene in an operon required for proliferation said method comprising:

(a) introducing into a cell a construct comprising an inducible fusion promoter operably linked to a nucleic acid that is complementary to at least a portion of a proliferation-required operon, said fusion promoter comprising at  
20 least one promoter that is modified to have altered activity in at least one gram-positive organism said promoter being linked to at least one operator selected from the group consisting of *xylo*, *tetO*, *trpO*, *malO* and *λclO*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter; and

25 (b) inducing transcription from said fusion promoter.

91. A method for inhibiting the activity or expression of a gene in an operon required for proliferation said method comprising:

30 (a) introducing into a cell a construct comprising an inducible fusion promoter operably linked to a nucleic acid that is complementary to at least a

portion of a proliferation-required operon, said fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59, P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *lacO*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter; and

(b) inducing transcription from said fusion promoter.

92. The method of Claim 90, wherein said at least one operator is *xylO*.

93. The method of Claim 92, wherein said at least one promoter is T5.

94. The method of Claim 92, wherein said fusion promoter further comprises a second operator.

95. The method of Claim 94, wherein said second operator is *lacO*.

96. The method of Claim 90, wherein said inducer is xylose.

97. The method of Claim 90, wherein said fusion promoter is titratable.

98. The method of Claim 90, wherein said at least one gram-positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.

99. The method of Claim 90, wherein said at least one gram-positive organism is *Staphylococcus aureus*.

100. The method of Claim 90, wherein said at least one gram-positive organism is *Enterococcus faecalis*.

101. A method of manufacturing an antibiotic comprising the steps of:

(a) introducing into a cell a construct comprising an inducible fusion promoter operably linked to a nucleic acid that is complementary to at least a portion of a proliferation-required gene, said fusion promoter comprising at

least one promoter that is modified to have altered activity in at least one gram-positive organism said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter;

(b) sensitizing said cell by inducing transcription from said fusion promoter;

(c) contacting said sensitized cell with a compound;

(d) identifying a compound which substantially inhibits the proliferation of said sensitized cell relative to a cell which has not been sensitized; and

(e) manufacturing the compound so identified.

102. A method of manufacturing an antibiotic comprising the steps of:

(a) introducing into a cell a construct comprising an inducible fusion promoter operably linked to a nucleic acid that is complementary to at least a portion of a proliferation-required gene, said fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59, P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter;

(b) sensitizing said cell by inducing transcription from said fusion promoter;

(c) contacting said sensitized cell with a compound;

(d) identifying a compound which substantially inhibits the proliferation of said sensitized cell relative to a cell which has not been sensitized; and

(e) manufacturing the compound so identified.

103. The method of Claim 101, wherein said at least one operator is *xylO*.

104. The method of Claim 103, wherein said at least one promoter is T5.
105. The method of Claim 103, wherein said fusion promoter further comprises a second operator.
106. The method of Claim 105, wherein said second operator is *lacO*.
- 5 107. The method of Claim 101, wherein said inducer is xylose.
108. The method of Claim 101, wherein said fusion promoter is titratable.
109. The method of Claim 101, wherein said at least one gram-positive organism is selected from the group consisting of *Bacillus anthracis*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*,  
10 *Corynebacterium diphtheriae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactococcus lactis*, *Listeria monocytogenes*, *Mycobacterium leprae*, *Mycobacterium tuberculosis*, *Nocardia asteroides*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylois*, *Streptococcus pneumoniae*, *Streptococcus mutans* and any species falling within the genera of any of the above species.
- 15 110. The method of Claim 101, wherein said at least one gram-positive organism is *Staphylococcus aureus*.
111. The method of Claim 101, wherein said at least one gram-positive organism is *Enterococcus faecalis*.
112. A method for identifying a gene which is required for proliferation of a  
20 prokaryotic cell said method comprising the steps of:
- (a) replacing the native promoter of a gene in the chromosome of a prokaryotic cell having an enhanced frequency of homologous recombination with a regulatable fusion promoter comprising at least one promoter that is modified to have altered activity in at least one gram-positive organism said  
25 promoter being linked to at least one operator selected from the group consisting of *xyiO*, *tetO*, *trpO*, *malO*, *lacO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter; and
- (b) identifying cells in which the extent of proliferation of said cell  
30 when said fusion promoter is active at a first level is substantially different

than the extent of proliferation of said cell when said fusion promoter is active at a second level, said first level being greater than said second level.

113. A method for identifying a gene which is required for proliferation of a prokaryotic cell said method comprising the steps of:

5 (a) replacing the native promoter of a gene in the chromosome of a prokaryotic cell having an enhanced frequency of homologous recombination with a regulatable fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59, P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of  
10 *xylO*, *tetO*, *trpO*, *malO*, *lacO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter; and

(b) identifying cells in which the extent of proliferation of said cell when said fusion promoter is active at a first level is substantially different  
15 than the extent of proliferation of said cell when said fusion promoter is active at a second level, said first level being greater than said second level.

114. The method of Claim 112, wherein said fusion promoter is an inducible promoter.

115. The method of Claim 112, wherein the step of replacing said native  
20 promoter comprises introducing a linear nucleic acid comprising a 5' portion homologous to a first portion of said native promoter, a 3' portion homologous to a second portion of said native promoter and said fusion promoter disposed between said 5' portion and said 3' portion into said cell such that homologous recombination occurs between said 5' portion and said first portion of said native promoter and  
25 between said 3' portion and said second portion of said native promoter.

116. The method of Claim 115, wherein said linear nucleic acid is double stranded.

117. The method of Claim 115, wherein said linear nucleic acid is single stranded.

118. A method for identifying a compound which inhibits the proliferation of a prokaryotic cell said method comprising the steps of:

5 (a) replacing the native promoter of a gene in the chromosome of a prokaryotic cell having an enhanced frequency of homologous recombination with a regulatable fusion promoter comprising at least one promoter that is modified to have altered activity in at least one gram-positive organism said promoter being linked to at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO*, *lacO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter; and

10 (b) comparing the extent of proliferation of a first sample of said cell in the presence of said compound to the extent of proliferation of a second sample of said cell in the presence of said compound, wherein said first sample of said cell has a reduced activity of said fusion promoter activity relative the activity of said fusion promoter in said second sample of said cell and wherein

15 said compound inhibits the proliferation of said cell if the extent of proliferation of said first sample of said cell is substantially less than the extent of proliferation of said second sample of said cell.

119. A method for identifying a compound which inhibits the proliferation of a prokaryotic cell said method comprising the steps of:

20 (a) replacing the native promoter of a gene in the chromosome of a prokaryotic cell having an enhanced frequency of homologous recombination with a regulatable fusion promoter comprising at least one promoter selected from the group consisting of T5, CP25, P32, P59, P1P2 and PL, said promoter being linked to at least one operator selected from the group consisting of

25 *xylO*, *tetO*, *trpO*, *malO*, *lacO* and *λc1O*, wherein said at least one operator is positioned such that binding of at least one repressor to said at least one operator represses transcription from said fusion promoter; and

30 (b) comparing the extent of proliferation of a first sample of said cell in the presence of said compound to the extent of proliferation of a second

sample of said cell in the presence of said compound, wherein said first sample of said cell has a reduced activity of said fusion promoter activity relative the activity of said fusion promoter in said second sample of said cell and wherein said compound inhibits the proliferation of said cell if the extent of proliferation of said first sample of said cell is substantially less than the extent of proliferation of said second sample of said cell..

120. The method of Claim 118, wherein the step of replacing said native promoter comprises introducing a linear nucleic acid comprising a 5' portion homologous to a first portion of said native promoter, a 3' portion homologous to a second portion of said native promoter and said fusion promoter disposed between said 5' portion and said 3' portion into said cell such that homologous recombination occurs between said 5' portion and said first portion of said native promoter and between said 3' portion and said second portion of said native promoter.

121. The method of Claim 120, wherein said linear nucleic acid is double stranded.

122. The method of Claim 120, wherein said linear nucleic acid is single stranded.

123. A method for identifying a gene which is required for proliferation of a prokaryotic cell said method comprising the steps of:

(a) introducing at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO*, *lacO* and *λc1O* into a prokaryotic cell having an enhanced frequency of homologous recombination such that said at least one operator regulates transcription of a target nucleic acid in the chromosome of said cell; and

(b) identifying cells in which the extent of proliferation of said cell when said target nucleic acid is transcribed at a first level is substantially different than the extent of proliferation of said cell when said target nucleic acid is transcribed at a second level, said first level being greater than said second level.



124. The method of Claim 123, wherein the step of introducing said at least one operator comprises introducing a linear nucleic acid comprising a 5' portion homologous to a first portion of the chromosome of said cell, a 3' portion homologous to a second portion of the chromosome of said cell and said at least one operator  
5 disposed between said 5' portion and said 3' portion into said cell such that homologous recombination occurs between the 5' portion and said first portion of the chromosome of said cell and between the 3' portion and said second portion of the chromosome of said cell.

125. The method of Claim 124, wherein said linear nucleic acid is double  
10 stranded.

126. The method of Claim 124, wherein said linear nucleic acid is single stranded.

127. A method of identifying a compound which inhibits the proliferation of a prokaryotic cell said method comprising the steps of:

15 (a) obtaining a prokaryotic cell in which transcription of a nucleic acid required for proliferation of said cell is regulated by at least one operator which has been introduced into the chromosome of said cell said at least one operator selected from the group consisting of *xylO*, *tetO*, *trpO*, *malO*, *lacO* and *λc1O*;

20 (b) sensitizing said cell by growing said cell under conditions in which the level of transcription of said gene is lower than that of a wild type cell;

(c) contacting said sensitized cell with said compound; and

(d) determining the degree to which said compound inhibits the growth of said sensitized cell relative to an unsensitized cell.

25 128. The method of Claim 127, wherein said cell has an enhanced frequency of homologous recombination.

129. The method of Claim 128, wherein said linear nucleic acid is single stranded.

30 130. The method of Claim 128, wherein said linear nucleic acid is double stranded.

131. A method of identifying a nucleic acid sequence having promoter activity in *Enterococcus faecalis* said method comprising the steps of:

(a) inserting a candidate nucleic acid into a vector comprising *lacL-lacM* reporter genes such that said candidate nucleic acid is upstream of the *lacL-lacM* reporter genes;

(b) introducing said vector comprising said candidate nucleic acid into *Enterococcus faecalis*;

(c) detecting expression of the *lacL-lacM* reporter genes, wherein expression of said *lacL-lacM* reporter genes indicates that said candidate nucleic acid sequence has promoter activity.

132. The method of Claim 131, wherein said detecting step comprises measuring  $\beta$ -galactosidase activity.

133. The method of Claim 131, wherein said candidate nucleic acid is a promoter modified to increase activity in a gram-positive organism.

134. The method of Claim 131, wherein said *lacL-lacM* reporter gene is derived from *Leuconostoc mesenteroides*.

135. The method of Claim 131, wherein said vector is pEPEF1.